Science Bulletin xxx (xxxx) xxx

Contents lists available at ScienceDirect

Science Bulletin

journal homepage: www.elsevier.com/locate/scib

Research Highlight

Non-genomic action of juvenile hormone modulates the synthesis of 20-hydroxyecdysone in *Drosophila*

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A wide array of biological events in the life history of insects are orchestrated by juvenile hormone (JH) and 20-hydroxyecdysone (20E) [1]. They control embryonic development, molting, metamorphosis, and reproduction. Some processes are governed by one of the two hormones while others rely on both. Larval development and metamorphosis in the tobacco hornworm, *Manduca sexta*, is a classic example manifesting the coordination of JH and 20E. 20E triggers every molt in *M. sexta*, from larval-larval molt to larval-pupal and pupal-adult molts; the nature of molting is defined by JH [2]. High levels of JH in the early larva instars exert the anti-metamorphic function and 20E initiates a molt that leads to another larger larva. Once the hornworm reaches a critical weight (a body size "check-point"), the synthesis of JH is halted. In the absence of JH, 20E instigates the metamorphic program, and the larva molts into the pupa [2]. Likewise, the egg maturation in the yellow fever mosquito *Aedes aegypti* is precisely regulated by JH and 20E. The oocyte development in female mosquitoes is controlled initially by JH after adult emergence. After a blood meal, JH levels drop rapidly and 20E becomes the major signal that commands the oocyte development [3]. The intricate interactions between JH and 20E remain poorly understood in insects. An article published in this issue reported a novel mechanism that JH uses to modulate 20E responses in *Drosophila melanogaster* [4].

Many JH actions in insects have been demonstrated to rely on its intracellular receptor, the methoprene-tolerant protein (Met) [5]. Upon the binding of JH, Met translocates to the nucleus and forms a protein complex with its DNA binding partner Taiman, acting as a transcriptional regulator to module gene expression [6,7]. JH signaling can also initiate from the cell membrane and the signal transduction involves the change of intracellular calcium levels and activation of several protein kinases, supporting the existence of a membrane JH receptor [8]. In the migratory locust *Locusta migratoria*, JH triggers rapid non-genomic action in follicle cells to form intercellular channels (referred to as "patency") within minutes to facilitate the uptake of vitellogenin in the hemolymph by the developing oocytes. It remains much unknown whether the cell membrane-initiated JH signaling plays a pivotal role in regulating insect development and metamorphosis. Gao et al. conducted a series of phosphoproteomic analyses to identify downstream targets that were post-translationally modified in fruit flies in response to the JH signal [4]. In *D. melanogaster*, Met and its homolog Germ cell-expressed (Gce) both function as the intracellular JH receptors [9]. To discern the Met/Gce-dependent and -independent phosphorylations, the authors designed a comparative analysis of phosphoproteomes of the JH-deficient flies, Met/Gce double null mutant, and the wild-type flies. An unexpected phosphorylation site that was consistently induced by a JH analog in a Met/Gce-independent manner was the serine 35 of ultraspiracle (USP), a component of the nuclear receptor complex for 20E [4].

After binding of 20E, the ecdysone receptor (EcR) forms a heterodimer with USP and often recruits Taiman as a transcriptional coactivator to activate the expression of 20E response genes [10]. In the newly emerged adult female *A. aegypti* mosquitoes, JH prompts the phosphorylation of serine/arginine-rich splicing factors, giving rise to specific Taiman isoforms that are required for the vitellogenic stage-specific 20E response after blood ingestion [11]. On the other hand, this study by Gao et al. demonstrated that JH alters the post-translational modification of a component of the 20E receptor complex through the cell membrane-initiated JH signal transduction and potentially modulates the function of the 20E receptor [4]. The phosphorylation of serine 35 is critically important for the transactivation activity of the EcR/USP complex [12], although the detailed molecular mechanism has not yet been elucidated. Using a gene knock-in strategy, the authors generated a transgenic fly line in which the serine 35 residual in the wild-type USP was replaced with an alanine. The mutant attenuated the expression of key 20E-inducible genes during the larval-prepupal transition, causing a delay in developmental timing and a considerable decrease in the pupal body size. The expression of several genes that are essential for 20E biosynthesis also displayed considerable downregulation compared with control flies, suggesting that the phosphorylation of USP regulates the production of 20E. This hypothesis was validated by the restoration of the delayed developmental timing of the usp^{S35A} mutant after dietary addition of 20E [4].

JH is known to act via Met/Gce and Krüppel homolog 1 (Kr-h1), a JH-inducible transcription factor, to inhibit the biosynthesis of ecdysone in the prothoracic gland in flies [13]. The new study discussed here thus provides an important addition to a holistic

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https://doi.org/10.1016/j.scib.2021.08.007

Please cite this article as: J. Zhu, Non-genomic action of juvenile hormone modulates the synthesis of 20-hydroxyecdysone in *Drosophila*, Science Bulletin, https://doi.org/10.1016/j.scib.2021.08.007

understanding of the JH regulation of 20E responses in flies. JH employs two signal transduction pathways to control the 20E action. The intracellular receptors of JH repress the 20E biosynthesis and Kr-h1 acts downstream of Met/Gce to suppress the expression of 20E response genes. In contrast, the cell membrane-initiated JH signaling promotes the production of 20E and presumably enhances the function of EcR/USP in activating 20-inducible genes. The two JH pathways seem to contribute to the homeostasis of ecdysone biosynthesis and ensure the precise timing of 20E responses [4]. It is important to note that the two JH pathways converge on Met-Taiman in the mosquitoes as the membrane signaling leads to the phosphorylation of both Met and Taiman to modulate the transcription of JH-regulated genes, such as Kr-h1 [14]. More work is needed to elucidate how the opposite actions of these two JH pathways are spatially and temporally coordinated.

The JH-induced phosphorylation of *Drosophila* USP requires the functions of receptor tyrosine kinases (RTK), phospholipase C (PLC), and protein kinase C (PKC) [4], consistent with the membrane-initiated JH signaling that was previously reported in *A. aegypti* mosquitoes during adult reproduction [14]. Activation of this pathway in the mosquitoes stimulates the phosphorylation of both Met and Taiman; the phosphorylation modifications are prerequisites for the binding of Met/Taiman to the regulatory regions of JH response genes. In addition, JH also acts via RTKs, phosphoinositide 3-kinase, and protein kinase B (Akt) in the mosquitoes to module stage-specific splicing of pre-mRNA [11]. The phosphoproteomic analysis of flies reported by Gao et al. unveiled several proteins that have annotated functions in mRNA splicing, suggesting that the pathway initiated from a putative membrane JH receptor is at least conserved in higher Diptera [4]. A similar JH signaling pathway is also found in the migratory locust *L. migratoria*, where JH triggers the development of ovarian follicular patency. The phosphorylation of the JH effector in *L. migratoria*, a Na^+/K^+-ATPase, was mediated by G protein-coupled receptors (GPCR), RTK, PLC, and PKC [15]. While the JH membrane signaling is similar in *L. migratoria* and *A. aegypti*, distinct differences still exist. The abovementioned JH membrane signaling in the mosquitoes does not require the participation of GPCRs. Different insects thus seem to have evolved to harness this JH membrane signaling to exert various JH regulatory functions in different settings. It is possible that multiple membrane proteins are involved and several downstream pathways are activated in a tissue- and stage-specific manner, depending on other cues and biological contexts. Additional comparative studies in the future will determine whether the JH-induced phosphorylation of Met takes place in insects other than *A. aegypti* and whether JH modulates the protein phosphorylation of USP in diverse insect orders.

JH and 20E are crucial regulators of many different biological processes in insects. The responses to each hormone vary at different stages and tissues. Therefore, the hormones are not a simple on-off control but can define and fine-tune specific responses by incorporating other signal inputs to generate distinctive timing and amplitude of the responses. One mechanism is adding extra layers of regulation. For example, post-transcriptional modification can form more sophisticated expression patterns than transcriptional regulation alone. Likewise, the membrane JH receptor and intracellular receptor make it possible to generate disparate responses to the same signal. While the membrane signaling and the Met-mediated transcriptional regulation can proceed independently, the convergence of both pathways leads to the phosphorylation of Met/Taiman, further enhancing the JH-induced gene expression [14]. The components involved in the membrane signaling may vary in abundance or activities in different biological settings, creating diverse hormone responses. Moreover, the JH membrane signaling encompasses the activation of common protein kinases and the change of second messengers, such as intracellular calcium, inositol trisphosphate (IP3), and diacylglycerol (DAG) [14]. The additional players open new opportunities for JH to interact with other cellular signal transduction pathways to impose far-reaching effects. The JH-triggered phosphorylation of USP is a perfect example of this cross-regulation between different hormones [4]. JH membrane signaling modulates stage-specific 20E responses by enhancing the expression of 20E-controlled genes and elevating the biosynthesis of 20E. These mechanisms together define the proper 20E response that is essential for normal development. Although it is not clear at this moment whether the JH-induced phosphorylation of USP is limited to a specific stage in flies, the finding reported in this paper represents major progress in recent years in our understanding of the molecular interaction between the JH and 20E signaling [4]. It opens up new avenues for the fundamental study of genetic control of insect development.

Conflict of interest

The author declares that he has no conflict of interest.

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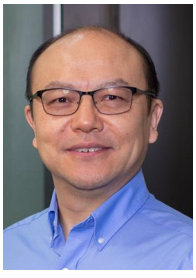

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